Cancer Therapy: Viral oncolysis.

As a post-doctoral fellow in the laboratory of John Holland I learned to grow tumor cells in the body cavity of mice. These were tumors that arose spontaneously in mice, had been grown in culture or maintained by passage through mice for many years, were easy to transplant, and grew rapidly. They were 100% lethal to the mice.. One held the mouse by the back of the neck, as one would pick up a cat, and injected the tumor into the body cavity. Within a few days the abdominal cavity puffed up like a balloon. It was rather horrible , and after a week to ten days the animal could barely move. The tumor cells were harvested by withdrawing them with a syringe from the body cavity, and cells could be kept " alive" by placing them into a flask with media. We used these cells for RNA isolation. In particular this approach worked with standard tumor cells lines such as Ehrlich Ascites Carcinoma (originally isolated by Paul Ehrlich) and sarcomas. We could harvest large amounts of cells, free of contamination.

I really do not remember how the idea arose to produce virus in these tumors after transplanting them into the animal. I thought it might be a way of reproducing the virus in large amounts without the need of cell culture. I myself did the original experiment, of injecting bovine enterovirus-1, which we happened to have handy (again brought with me from the Holland lab), and injecting the virus into a few animals. To my surprise the tumors regressed within a day or so and the mice returned to a healthy appearance. I was very excited by this discovery and felt I had made an important novel scientific discovery. A cure for cancer! The magic bullet! I decided this was worth pursuing further and asked an undergraduate (JD and now a retired professor at Lexington Kentucky) working on an independent project to test this further, and discussed the phenomenon with some of the my graduate students. We then tested the virus, (BEV) against a large number of tumor cell lines. In all cases, both in vivo (in the mouse) and in vitro (cell culture) the virus destroyed the tumors.

Quite a number of students were involved in this work, including Suzanne Prather, the wife of Larry Prather who was the department " tech" person. While studying for the Ph.D. she was diagnosed with breast cancer. After receiving her Ph.D. she took a position at

the University of Nebraska, but died a few years later. She was a very strong willed person, not wanting to give up, and fought her cancer vigorously, suffering through many surgeries. She would come to the lab on crutches as the cancer spread.

Others involved in this work include Gerry Sedmak, a red headed graduate student, who was difficult to distinguish from his twin brother, who was also in the department. The brother, James (Jim) went on to work in the field of interferon. I probably have met them both at scientific meetings, not able to tell them apart. Others working on this project were Barbara Cordell_Stewart, and a visiting physician Magda Souhrada. Magda had recently arrived from Czechoslovakia, having fled after the Soviet invasion of the country. Her husband had found a job in the physics department, and although a MD she could not work in that capacity until she passed exams to practice medicine. After a year or so she did begin working with Dr Riley Schaffer, a local pediatrician. .Barbara was a graduate student.

A perusal of scientific–cancer literature indicated that this unfortunately was not a novel finding but an old phenomenon, reported many years ago. Viruses of various origins were used to treat cancer in both the US and in Japan going back to the 1940's. This was based on an observation made around the 1900's that some patients with cancers went into remission after a viral infection or rabies vaccination. At that time viruses were still unknown entities. It was not clear in the 1940's whether this resulted from activation of the immune response or due to direct action of the virus on the tumor, or a combination of both. A large body of research was performed in the US in the 1940's and 1950's testing various viruses, particularly exotic ones, that is viruses that occurred predominantly in the tropics, on terminal cancer patients ,with only limited results. Most patients died, but there was some objective regression of tumor mass. These patients were terminally ill when treatment was initiated. .This research was discredited in the 1950's when it was found that patients in one hospital had been injected with cancer cells to test the effect of various anti-cancer agents including virus. In most cases the injected tumors were rejected by the immune system, although occasionally they had to be surgically removed. The researcher involved was later criticized for this type of work, since he had not gone through the normal protocols for protection of human subjects even at that time. I am not certain there was a human subjects committee.

A group in Japan used mumps virus to treat a large number of patients with some transient effect on tumor growth. There was some limited success with the prolongation of life of terminal cancer patients, but the treatment was not performed in sufficient numbers to be statistically significant. The experiments in Japan, although performed better than those done in the US had very mixed results. It was obvious that before this could proceed to the clinic there was need for considerable basic research.

I therefore asked some graduate students and undergraduates to work out the basics: and we decided to use BEV-1 as a model viral system. We published a number of papers on viral oncolysis, or virotherapy as this phenomenon is now called, in influential journals (Nature New Biology, PNAS). BEV-1 destroyed tumors of various kinds, not only ascites tumors and not only in mice. In collaboration with Dr. E. Hodes at the Medical School we showed that the virus also destroyed tumors of rabbits and dogs. If we could obtain the funding we were set to begin clinical trials. However I, and others working in viral oncolysis, found it difficult to obtain grants. The idea that viruses could be used as therapeutic agents in the 1970s was way before its time. Until very recently is was difficult to get funded by the NIH for this type of work.

When we published our papers on viral oncolysis, the local and national press picked up the story, after a press release by the IU news department. I received letters and phone calls from as far away as Australia asking for the virus to treat tumors in dogs and in humans. I constantly had to point out that this was an experimental system. I had a visit from two scientists from Japan, Dr. Teruo Asada and Dr. Yamanishsi from Osaka University, to consult and discuss their results with mumps virus. The Japanese scientists came laden with presents. One of my colleagues Bob Togasaki acted as interpreter. I just noted on Dr. Asada's paper in Cancer, 1974, that he thanked myself, and Dr. Hodes of Indianapolis as well as Dr. Togasaki for discussions of the work. I remember that we hosted them at home, Mimi prepared an elegant dinner (as usual) and they came laden with presents. It was a bit like Xmas, presents for Mimi (pearls), presents for the house, for Dr. and Mrs. Togasaki. I did not quite know how to respond, but Bob Togasaki's advice was not to reciprocate otherwise the exchange of presents would go on forever. In a way this was my moment of

fame. The work was quoted in many sources. I received acclaim from the American Cancer Society, and I found it very hard to let go.

However because of lack of funding viral oncolysis lay dormant as a project for quite a number of years and I became involved much more with other projects. One can question my judgment, in whether it was wise to give this up, or in fact would have been better to re-apply for funding and continue the work. My attitude, which in hindsight was probably wrong, was to give up on a project that was not funded the first or second time around. I have learned from my colleagues and having been on many grant study sections, that it may take multiple re-writes to satisfy everyone on a study section committee, and to " knock" the score up by a few points so that it is within the fundable range. However at that time there seemed no future in viral oncolysis. I went for a job interview at the university of Georgia in the late 1970's (I am not certain I would have even moved), but although my talk went down well, my host, the chairman of the department took me aside and told me he thought I was working in the wrong area. An area without a future. He may have been correct, but it is difficult to give up an idea that seemed so promising. Today I am much more skeptical about research in general, certainly as to its applications. Basic research for knowledge sake is something else.

Today, fifty years later experiments treating cancer with a virus is still being pursued and in China viruses (adenovirus and herpes viruses) are used in the clinic in combination with chemotherapy or other treatments. Quite a number of biotech companies have been launched with this idea, and just as many have folded. Amgen, the company I worked with in the 1990's has just bought out another biotech company B-vec for 1 billion dollars to explore viral oncolysis. Currently a modified herpes virus containing a gene associated with the immune system (GM-CSF) is undergoing phase 3 trials for the treatment of melanoma and head and neck cancer. The reported rate of remission for melanoma is 25% with the virus containing the GM-CSF gene and only 2% using GM-CSF alone. Other oncolytic-modified viruses are being tested in the presence of chemotherapeutic agents with some success. It appears that the obstacles to viral therapy are still the ones we discussed in the 1970's. These include how to get the virus to the target, the immune and interferon response against the virus, the development of antibodies against the virus, and possible

adverse effects of the virus. These same obstacles have hindered progress in gene therapy, and are difficult to overcome. However viruses have been engineered, by deleting or adding genes, sometimes from non-viral sources that overcome many of these difficulties.

Viral oncolysis reappeared again in my own career many years later when we did experiments using another virus (adenovirus) in gene therapy experiments using a virus carrying an interferon gene. That is a separate story under the heading **gene therapy.** Perhaps I should go back to BEV, modify the virus, and restart my career.